Author’s response to reviews

Title: Genetic and biochemical markers of hydroxyurea therapeutic response in sickle cell anemia

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Author’s response to reviews: see over
I write on behalf my supervisor and corresponding author Eduardo Alves de Almeida.

First, we would like to thank the reviewers for detailed review and very well argued. All aspects and concerns raised to article improvement were taken into consideration and we hope to answer all the doubts in order enlightening.

All changes are highlighted with red color, in both text structure as well English corrections.

Attached, you will find a article file with the changes made and answers to referees doubts follow below.

Reviewer’s report (Philippe Joly)

Major Compulsory Revisions

We appreciate and agree with the quality of Rees and Gibson work which reinforces many different SCD biomarkers that have been described in literature. However, most of these biomarkers are of limited clinical value. Rees and Gibson also cited a biomarker definition according to The Biomarkers Definitions Working Group (2001): ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention’. And oxidative stress biomarkers appear to exhibit such predicates.

Even Ware et al (2011) concluded that accurate prediction of hydroxyurea treatment responses for SCA remains a worthy but elusive goal. But our article did not seek to identify genetic and biochemical markers of response to hydroxyurea (hydroxycarbamide) therapy for SCA. But we found some relationship between the chosen markers that suggest different responses among de beta S globin haplotypes, according with biochemical parameters evaluated.

We continue studying the beta-S globin haplotypes with larger sample size and sample groups similar to those suggested to better address the present hypothesis.

In Brazil, SCA patients are treated according to Ministry of Health Ordinance No 872 from November 6 2002, Entitled “Therapeutic Clinical Guideline - Disease Sickle Cell -
Hydroxyurea”. This guidelines recommends initial dose of 15 mg/kg/day, once a day. This dose can be increased to 5 mg/kg/day every eight to 12 weeks, with the aim is to achieve the maximum tolerated dose (MTD) and MTD should not exceed 35 mg/kg/day. However, SCA patients evaluated in this study already showed prominently and satisfactory improves of clinical and laboratory course of the disease with a mean dose of 20 mg/kg/day. Thus, MTD was not tested and applied.

Minor Essential Revisions were fulfilled.

Observation: In the Table VI, the letters were used to show at a simplistic way, statistical differences between groups. So groups which present same letters did not differ statistically.

Reviewer’s report (Frederic Galacteros)

I agree with "small sample size", however Brazilian population derives from many ethnic and national origins and includes native American Indian people, Europeans of Portuguese, Italian and German origin, Japanese and African people. Combinations of βS gene with β-Thalassemia genes of Italian origin reduces Hb SS frequency, specially in Sao Paulo state where we are located. We first analyzed 70 sickle cell disease patients, after molecular genotype confirmation, 18 were characterized as Hb S/Beta Thalassemia and 52 as HB SS. Of those 52 ones, only 28 SCA patients fulfilled inclusion criteria adopted in this study.

Even then, the small sample does not refute the hypothesis or even decreases the importance and uniqueness of these results. Rather, the small sample size leaves us a prospect of new and more studies with larger sample size to better address this hypothesis.

Minor issues:

- We emphasize that our article did not seek to identify new markers of response to HU therapy for sickle cell anemia. For this reason, such biological markers of HU effects have not been evaluated.

- we believe there was a misunderstood in relationship to the Figure 1c. This figure does not show any comparisons between patient's populations. It shows an association degree between two markers evaluated (Hb F and lipid peroxidation levels), corroborating their levels presented in Figure 1a and 1b. In other words, high Hb F levels in SCA patients culminate in low lipid peroxidation.
- Please, review the subheading 'Hemoglobin phenotypes, genotypes and βS-globin haplotypes' where we described cytological, electrophoretic, chromatographic and molecular methods used to confirm HbSS genotype.

We tried to avoid many biases that could interfere in the biochemical markers evaluated, but alpha thalassemia trait screening have not been aimed.

Reviewer’s report (Umberto Moscato)

Discretionary Revisions

1) This kind of symbology (< or > and =) is very common in our field. We never saw the suggested symbol (#) in the papers from our field. Even in the published manuscripts in the BMC Medical Genetics used the same symbols we used.

Minor Essential Revisions were fulfilled and we appreciated the comments and corrections.

Major Compulsory Revisions

1) Statistical Analysis (general considerations):

In our field it is extremely unusual to do these kind of statistics methods suggested, such as study power or minimal sample size. We obtained an unexpected and not well documented in the literature response to hydroxyurea treatment. So, we had none previous study to give us subsidies to better define sample size or which statistic analysys to use.

We have none statistic or biostatistics expert in our work group, but to best of our knowledge there are some characteristics to maximizing statistical/study power, like increase sample size, increase alpha level, select reliable measures for the study, develop a strong research design and use all information available to identify an appropriate population and recruit participants for the study. And we did that. It is not easy to increase sample number of SCA patients with the genetic
characteristics evaluated in this study and inclusion criteria adopted (see comments to Frederic Galacteros).

Could we be making a Type II error? We can never know for certain, but, if we calculate our effect size, we could get a clue. However, of the factors that affect statistical power, the only one not under the direct control of the researcher is effect size.

Another interesting observation is that we would try to increase the sample size when we are not able to reject a false null hypothesis. Thus, we can believe that with a larger patients number, a statistical difference obtained would be even higher.

We rewrite statistical analyzes to make sure they are correct and understandable. We emphasize that all the analysis was done according to the book by Gerry P. Quinn and Michael J. Keough "Experimental Design and Data Analysis for Biologists" (2002) and that the tests used are widespread to the field papers.

Once again, I would like to thank you for attention.

My best regards,

Danilo.

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